(FILE 'HOME' ENTERED AT 14:31:40 ON 11 APR 2003)

FILE 'MEDLINE, AGRICOLA, CANCERLIT, SCISEARCH, CAPLUS, MEDICONF' ENTERED AT 14:31:50 ON 11 APR 2003

L1 398 S HS? (L) ZETA?
L2 222 DUP REM L1 (176 DUPLICATES REMOVED)
L3 21 S L2 AND HS-40
L4 21 SORT L3 PY
L5 5 S L1 AND RETROVIR?
L6 3 DUP REM L5 (2 DUPLICATES REMOVED)
L7 3 SORT L6 PY
L8 12 S L3 AND PROMOT?
L9 9 S L8 AND ENHANC?
E SHEN JAMES? /AU

=> d an ti so au ab pi l9 9

L9 ANSWER 9 OF 9 CAPLUS COPYRIGHT 2003 ACS
AN 2001:757858 CAPLUS
DN 135:314417
TI Vectors containing mutated **HS-40 enhancer** of .**zeta**.-globin gene **promoter** and its regulation of transgene expression in transgenic mice
SO U.S., 7 pp., Division of U.S. Ser. No. 205,015, abandoned.
CODEN: USXXAM
IN Shen, Che-Kun James
AB The invention relates to a mutated **HS-40 enhancer** of .**zeta**.-globin gene **promoter**, a 350-400 bp **enhancer** element located about 40 kb upstream of . **zeta**.-globin gene. **HS-40** is the major cis-acting regulatory element responsible for the developmental stage-and erythroid lineage-specific expression of the human .alpha.-like globin genes, the embryonic .**zeta**. and the adult .alpha.2/.alpha./1. The invention is based on the discovery that a single nucleotide change in the 3'NF-E2/AP1 element of the human **HS-40 enhancer**, unlike the wild type **HS-40 enhancer**, confers position-independent and copy no.-dependent expression on a transgene. In addn., the single nucleotide change allows expression of the gene in the cells of an adult mouse, an effect not seen for the wild type **HS-40 enhancer**. Accordingly, the invention provides a viral expression vector (e.g., a retrovirus) expressing a transgene regulated by (1) a transcriptional start site; (2) a **promoter** (e.g., a tissue-specific **promoter** such as .**zeta**.-globin **promoter**) operably linked to the transcriptional start site; and (3) the above mutated **HS-40 enhancer** operably linked to the **promoter**.

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	US 6303845	B1	20011016	US 2000-536094	20000324
	US 2002108134	A1	20020808	US 2001-977432	20011015

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L9 9 S L8 AND ENHANC?
E SHEN JAMES? /AU

=> d an ti so au ab pi l9 1-9

L9 ANSWER 1 OF 9 MEDLINE
AN 2000437243 MEDLINE
TI Non-erythroid genes inserted on either side of human **HS-40** impair the activation of its natural alpha -globin gene targets without being themselves preferentially activated.
SO JOURNAL OF BIOLOGICAL CHEMISTRY, (2000 Aug 18) 275 (33) 25831-9. Journal code: 2985121R. ISSN: 0021-9258.
AU Esperet C; Sabatier S; Deville M A; Ouazana R; Bouhassira E E; Godet J; Morle F; Bernet A
AB The human alpha-globin gene complex includes three functional globin genes (5'-**zeta2**-alpha2-alpha1-3') regulated by a common positive regulatory element named **HS-40** displaying strong erythroid-specific **enhancer** activity. How this **enhancer** activity can be shared between different **promoters** present at different positions in the same complex is poorly understood. To address this question, we used homologous recombination to target the insertion of marker genes driven by cytomegalovirus or long terminal repeat **promoters** in both possible orientations either upstream or downstream from the **HS-40** region into the single human alpha-globin gene locus present in hybrid mouse erythroleukemia cells. We also used CRE recombinase-mediated cassette exchange to target the insertion of a tagged alpha-globin gene at the same position downstream from **HS-40**. All these insertions led to a similar decrease in the **HS-40**-dependent transcription of downstream human alpha-globin genes in differentiated cells. Interestingly, this decrease is associated with the strong activation of the proximal newly inserted alpha-globin gene, whereas in marked contrast, the transcription of the non-erythroid marker genes remains insensitive to **HS-40**. Taken together, these results indicate that the **enhancer** activity of **HS-40** can be trapped by non-erythroid **promoters** in both upstream and downstream directions without necessarily leading to their own activation.

L9 ANSWER 2 OF 9 MEDLINE
AN 2000153760 MEDLINE
TI Loading of DNA-binding factors to an erythroid **enhancer**.
SO MOLECULAR AND CELLULAR BIOLOGY, (2000 Mar) 20 (6) 1993-2003. Journal code: 8109087. ISSN: 0270-7306.
AU Wen S C; Roder K; Hu K Y; Rombel I; Gavva N R; Daftari P; Kuo Y Y; Wang C; Shen C K
AB The **HS-40 enhancer** is the major cis-acting regulatory element responsible for the developmental stage- and erythroid lineage-specific expression of the human alpha-like globin genes, the embryonic **zeta** and the adult alpha2/alpha/1. A model has been proposed in which competitive factor binding at one of the **HS-40** motifs, 3'-NA, modulates the capability of **HS-40** to activate the embryonic **zeta**-globin **promoter**. Furthermore, this modulation was thought to be mediated through configurational changes of the **HS-40** **enhanceosome** during development. In this study, we have further investigated the molecular basis of this model. First, human erythroid K562 cells stably integrated with various **HS-40** mutants cis linked to a human alpha-globin **promoter**-growth

hormone hybrid gene were analyzed by genomic footprinting and expression analysis. By the assay, we demonstrate that factors bound at different motifs of **HS-40** indeed act in concert to build a fully functional **enhanceosome**. Thus, modification of factor binding at a single motif could drastically change the configuration and function of the **HS-40 enhanceosome**. Second, a specific 1-bp, GC-->TA mutation in the 3'-NA motif of **HS-40**, 3'-NA(II), has been shown previously to cause significant derepression of the embryonic **zeta**-globin **promoter** activity in erythroid cells. This derepression was hypothesized to be regulated through competitive binding of different nuclear factors, in particular AP1 and NF-E2, to the 3'-NA motif. By gel mobility shift and transient cotransfection assays, we now show that 3'-NA(II) mutation completely abolishes the binding of small MafK homodimer. Surprisingly, NF-E2 as well as AP1 can still bind to the 3'-NA(II) sequence. The association constants of both NF-E2 and AP1 are similar to their interactions with the wild-type 3'-NA motif. However, the 3'-NA(II) mutation causes an approximately twofold reduction of the binding affinity of NF-E2 factor to the 3'-NA motif. This reduction of affinity could be accounted for by a twofold-higher rate of dissociation of the NF-E2-3'-NA(II) complex. Finally, we show by chromatin immunoprecipitation experiments that only binding of NF-E2, not AP1, could be detected in vivo in K562 cells around the **HS-40** region. These data exclude a role for AP1 in the developmental regulation of the human alpha-globin locus via the 3'-NA motif of **HS-40** in embryonic/fetal erythroid cells. Furthermore, extrapolation of the in vitro binding studies suggests that factors other than NF-E2, such as the small Maf homodimers, are likely involved in the regulation of the **HS-40** function in vivo.

L9 ANSWER 3 OF 9 MEDLINE
AN 1999061925 MEDLINE
TI Derepression of human embryonic zeta-globin **promoter** by a locus-control region sequence.
SO PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1998 Dec 8) 95 (25) 14669-74.
Journal code: 7505876. ISSN: 0027-8424.
AU Huang B L; Fan-Chiang I R; Wen S C; Koo H C; Kao W Y; Gavva N R; Shen C K
AB A multiple protein-DNA complex formed at a human alpha-globin locus-specific regulatory element, **HS-40**, confers appropriate developmental expression pattern on human embryonic **zeta**-globin **promoter** activity in humans and transgenic mice. We show here that introduction of a 1-bp mutation in an NF-E2/AP1 sequence motif converts **HS-40** into an erythroid-specific locus-control region. Cis-linkage with this locus-control region, in contrast to the wild-type **HS-40**, allows erythroid lineage-specific derepression of the silenced human **zeta**-globin **promoter** in fetal and adult transgenic mice. Furthermore, **zeta**-globin **promoter** activities in adult mice increase in proportion to the number of integrated DNA fragments even at 19 copies/genome. The mutant **HS-40** in conjunction with human **zeta**-globin **promoter** thus can be used to direct position-independent and copy number-dependent expression of transgenes in adult erythroid cells. The data also supports a model in which competitive DNA binding of different members of the NF-E2/AP1 transcription factor family modulates the developmental stage specificity of an erythroid **enhancer**. Feasibility to reswitch on embryonic/fetal globin genes through the manipulation of nuclear factor binding at a single regulatory DNA motif is discussed.

L9 ANSWER 4 OF 9 MEDLINE
AN 97351165 MEDLINE
TI Analysis of **enhancer** function of the **HS-40** core sequence of the human alpha-globin cluster.
SO NUCLEIC ACIDS RESEARCH, (1997 Jul 15) 25 (14) 2917-22.
Journal code: 0411011. ISSN: 0305-1048.
AU Chen H; Lowrey C H; Stamatoyannopoulos G
AB **HS-40** is the major regulatory element of the human alpha-globin locus, located 40 kb upstream of the **zeta**-globin gene. To test for potential interactions between **HS-40**

and the beta- or the gamma-globin gene **promoters** in stable transfection assays, the **HS-40** core sequence was cloned upstream of either the beta **promoter** or the gamma **promoter** driving the neomycin phosphotransferase gene and **enhancer** activity was measured using a colony assay. In K562 or in MEL cells, **enhancer** activity of **HS-40** was higher than that of the individual core sequences of the DNase I hypersensitive sites (**HS**) of the beta-globin locus control region (LCR), and approximately 60% of the **enhancer** activity of a 2.5 kb microLCR, which contains the core elements of DNase I hypersensitive sites 1-4. In contrast to the synergistic interaction between the DNase I hypersensitive sites of beta locus LCR, combination of **HS-40** with these DNase I hypersensitive sites failed to display cooperativity in K562 cells and inhibited **enhancer** function in MEL cells. Inhibition of **enhancer** function was also observed when two copies of the **HS-40** were arranged tandemly. We conclude that the core element of **HS-40** (i) is a powerful **enhancer** of gamma- and beta-globin gene expression, (ii) in contrast to other classical **enhancers**, acts best as a single copy, (iii) does not cooperate with the regulatory elements of the beta-globin locus control region.

L9 ANSWER 5 OF 9 MEDLINE
AN 95327665 MEDLINE
TI Transcriptional activation of human adult alpha-globin genes by hypersensitive site-40 **enhancer**: function of nuclear factor-binding motifs occupied in erythroid cells.
SO PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1995 Jul 3) 92 (14) 6454-8.
Journal code: 7505876. ISSN: 0027-8424.
AU Rombel I; Hu K Y; Zhang Q; Papayannopoulou T; Stamatoyannopoulos G; Shen C K
AB The developmental stage- and erythroid lineage-specific activation of the human embryonic **zeta**- and fetal/adult alpha-globin genes is controlled by an upstream regulatory element [hypersensitive site (**HS**)-**40**] with locus control region properties, a process mediated by multiple nuclear factor-DNA complexes. In vitro DNase I protection experiments of the two G+C-rich, adult alpha-globin **promoters** have revealed a number of binding sites for nuclear factors that are common to HeLa and K-562 extracts. However, genomic footprinting analysis has demonstrated that only a subset of these sites, clustered between -130 and +1, is occupied in an erythroid tissue-specific manner. The function of these in vivo-occupied motifs of the alpha-globin **promoters**, as well as those previously mapped in the **HS-40** region, is assayed by site-directed mutagenesis and transient expression in embryonic/fetal erythroid K-562 cells. These studies, together with our expression data on the human embryonic **zeta**-globin **promoter**, provide a comprehensive view of the functional roles of individual nuclear factor-DNA complexes in the final stages of transcriptional activation of the human alpha-like globin **promoters** by the **HS-40** element.

L9 ANSWER 6 OF 9 MEDLINE
AN 95244883 MEDLINE
TI Identification of a major positive regulatory element located 5' to the human zeta-globin gene.
SO BLOOD, (1995 May 1) 85 (9) 2587-97.
Journal code: 7603509. ISSN: 0006-4971.
AU Sabath D E; Koehler K M; Yang W Q; Patton K; Stamatoyannopoulos G
AB The function of the **zeta**-globin **promoter** was studied using a series of **zeta**-globin **promoter** deletion constructs to drive luciferase expression in transiently transfected human erythroleukemia cells. The **promoters** were used without **enhancers**, or with **enhancers** derived from the beta-globin locus control region and the alpha-globin **HS-40 enhancer**. When transfected into K562 cells, which express **zeta**-globin, comparable amounts of activity were obtained from the -557 and -417 **zeta**-luciferase constructs and the alpha-luciferase constructs when no **enhancers** or the alpha-globin locus **enhancers** were used. When the constructs were

transfected into OCIM1 cells, which do not express **zeta**-globin, the **zeta**-globin **promoters** were at best 20% as active as the alpha-globin **promoters**. When sequences from -417 to -207 5' to the **zeta**-globin mRNA cap site were deleted, up to 95% of the **zeta**-globin **promoter** activity was lost in K562 cells. Reinsertion of these sequences into **zeta**-luciferase constructs missing the -417 to -207 region showed that the sequences lack classical **enhancer** activity. Point mutation of a GATA-1 site at -230 reduced **promoter** activity by 37%. Point mutation of a CCACC site at -240 had no effect. Electrophoretic mobility shift assays indicated that the -230 GATA-1 site has a relatively low affinity for GATA-1. These experiments show the presence of a strong positive-acting element, located between -417 and -207 bp 5' to the **zeta**-globin mRNA cap site, is necessary for high-level **promoter** activity in K562 cells. This element requires GATA-1 and additional unknown factors for maximal activity.

L9 ANSWER 7 OF 9 MEDLINE
AN 95238333 MEDLINE
TI Functional roles of in vivo footprinted DNA motifs within an alpha-globin **enhancer**. Erythroid lineage and developmental stage specificities.
SO JOURNAL OF BIOLOGICAL CHEMISTRY, (1995 Apr 14) 270 (15) 8501-5. Journal code: 2985121R. ISSN: 0021-9258.
AU Zhang Q; Rombel I; Reddy G N; Gang J B; Shen C K
AB Transcriptional regulation of the human alpha-like globin genes, embryonic **zeta** 2 and adult alpha, during erythroid development is mediated by a distal **enhancer**, **HS-40**. Previous protein-DNA binding studies have shown that **HS-40** consists of multiple nuclear factor binding motifs that are occupied in vivo in an erythroid lineage- and developmental stage-specific manner. We have systematically analyzed the functional roles of these factor binding motifs of **HS-40** by site-directed mutagenesis and transient expression assay in erythroid cell cultures. Three of these **HS-40 enhancer** motifs, 5'NF-E2/AP1, GT II, and GATA-1(c), positively regulate the **zeta** 2-globin **promoter** activity in embryonic/fetal erythroid K562 cells and the adult alpha-globin **promoter** activity in adult erythroid MEL cells. On the other hand, the 3'NF-E2/AP1 motif is able to exert both positive and negative regulatory effects on the **zeta** 2-globin **promoter** activity in K562 cells, and this dual function appears to be modulated through differential binding of the ubiquitous AP1 factors and the erythroid-enriched NF-E2 factor. Mutation in the GATA-1(d) motif, which exhibits an adult erythroid-specific genomic footprint, decreases the **HS-40 enhancer** function in dimethyl sulfoxide-induced MEL cells but not in K562 cells. These studies have defined the regulatory roles of the different **HS-40** motifs. The remarkable correlation between genomic footprinting data and the mutagenesis results also suggests that the erythroid lineage- and developmental stage-specific regulation of human alpha-like globin **promoters** is indeed modulated by stable binding of specific nuclear factors in vivo.

L9 ANSWER 8 OF 9 MEDLINE
AN 93204975 MEDLINE
TI Transcriptional activation of human **zeta** 2 globin **promoter** by the alpha globin regulatory element (**HS-40**): functional role of specific nuclear factor-DNA complexes.
SO MOLECULAR AND CELLULAR BIOLOGY, (1993 Apr) 13 (4) 2298-308. Journal code: 8109087. ISSN: 0270-7306.
AU Zhang Q; Reddy P M; Yu C Y; Bastiani C; Higgs D; Stamatoyannopoulos G; Papayannopoulou T; Shen C K
AB We studied the functional interaction between human embryonic **zeta** 2 globin **promoter** and the alpha globin regulatory element (**HS-40**) located 40 kb upstream of the **zeta** 2 globin gene. It was shown by transient expression assay that **HS-40** behaved as an authentic **enhancer** for high-level **zeta** 2 globin **promoter** activity in K562 cells, an erythroid cell line of embryonic and/or fetal origin. Although sequences located between -559 and -88 of the **zeta** 2 globin gene were dispensable for its expression on **enhancerless** plasmids, they

were required for the **HS-40 enhancer** -mediated activity of the **zeta** 2 globin **promoter**. Site-directed mutagenesis demonstrated that this **HS-40 enhancer-zeta** 2 globin **promoter** interaction is mediated by the two GATA-1 factor binding motifs located at -230 and -104, respectively. The functional domains of **HS-40** were also mapped. Bal 31 deletion mapping data suggested that one GATA-1 motif, one GT motif, and two NF-E2/AP1 motifs together formed the functional core of **HS-40** in the erythroid-specific activation of the **zeta** 2 globin **promoter**. Site-directed mutagenesis further demonstrated that the **enhancer** function of one of the two NF-E2/AP1 motifs of **HS-40** is mediated through its binding to NF-E2 but not AP1 transcription factor. Finally, we did genomic footprinting of the **HS-40 enhancer** region in K562 cells, adult nucleated erythroblasts, and different nonerythroid cells. All sequence motifs within the functional core of **HS-40**, as mapped by transient expression analysis, appeared to bind a nuclear factor(s) in living K562 cells but not in nonerythroid cells. On the other hand, only one of the apparently nonfunctional sequence motifs was bound with factors in vivo. In comparison to K562, nucleated erythroblasts from adult human bone marrow exhibited a similar but nonidentical pattern of nuclear factor binding in vivo at the **HS-40** region. These data suggest that transcriptional activation of human embryonic **zeta** 2 globin gene and the fetal/adult alpha globin genes is mediated by erythroid cell-specific and developmental stage-specific nuclear factor-DNA complexes which form at the **enhancer** (**HS-40**) and the globin **promoters**.

L9 ANSWER 9 OF 9 CAPLUS COPYRIGHT 2003 ACS
AN 2001:757858 CAPLUS
DN 135:314417
TI Vectors containing mutated **HS-40 enhancer** of .**zeta**.-globin gene **promoter** and its regulation of transgene expression in transgenic mice
SO U.S., 7 pp., Division of U.S. Ser. No. 205,015, abandoned.
CODEN: USXXAM
IN Shen, Che-Kun James
AB The invention relates to a mutated **HS-40 enhancer** of .**zeta**.-globin gene **promoter**, a 350-400 bp **enhancer** element located about 40 kb upstream of .**zeta**.-globin gene. **HS-40** is the major cis-acting regulatory element responsible for the developmental stage-and erythroid lineage-specific expression of the human .alpha.-like globin genes, the embryonic .**zeta**. and the adult .alpha.2/.alpha./1. The invention is based on the discovery that a single nucleotide change in the 3'NF-E2/AP1 element of the human **HS-40 enhancer**, unlike the wild type **HS-40 enhancer**, confers position-independent and copy no.-dependent expression on a transgene. In addn., the single nucleotide change allows expression of the gene in the cells of an adult mouse, an effect not seen for the wild type **HS-40 enhancer**. Accordingly, the invention provides a viral expression vector (e.g., a retrovirus) expressing a transgene regulated by (1) a transcriptional start site; (2) a **promoter** (e.g., a tissue-specific **promoter** such as .**zeta**.-globin **promoter**) operably linked to the transcriptional start site; and (3) the above mutated **HS-40 enhancer** operably linked to the **promoter**.

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	US 2002108134	A1	20020808	US 2001-977432	20011015

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L4 ANSWER 20 OF 21 CAPLUS COPYRIGHT 2003 ACS
AN 2001:757858 CAPLUS
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TI Vectors containing mutated **HS-40** enhancer of . **zeta**.-globin gene promoter and its regulation of transgene expression in transgenic mice
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AB The invention relates to a mutated **HS-40** enhancer of . **zeta**.-globin gene promoter, a 350-400 bp enhancer element located about 40 kb upstream of .**zeta**.-globin gene. **HS-40** is the major cis-acting regulatory element responsible for the developmental stage-and erythroid lineage-specific expression of the human .alpha.-like globin genes, the embryonic .**zeta**. and the adult .alpha.2/.alpha./1. The invention is based on the discovery that a single nucleotide change in the 3'NF-E2/AP1 element of the human **HS-40** enhancer, unlike the wild type **HS-40** enhancer, confers position-independent and copy no.-dependent expression on a transgene. In addn., the single nucleotide change allows expression of the gene in the cells of an adult mouse, an effect not seen for the wild type **HS-40** enhancer. Accordingly, the invention provides a viral expression vector (e.g., a retrovirus) expressing a transgene regulated by (1) a transcriptional start site; (2) a promoter (e.g., a tissue-specific promoter such as .**zeta**.-globin promoter) operably linked to the transcriptional start site; and (3) the above mutated **HS-40** enhancer operably linked to the promoter.

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
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US 2002108134	A1	20020808	US 2001-977432	20011015

L4 ANSWER 1 OF 21 MEDLINE
AN 91342671 MEDLINE
TI Characterization of the major regulatory element upstream of the human alpha-globin gene cluster.
SO MOLECULAR AND CELLULAR BIOLOGY, (1991 Sep) 11 (9) 4679-89.
Journal code: 8109087. ISSN: 0270-7306.
AU Jarman A P; Wood W G; Sharpe J A; Gourdon G; Ayyub H; Higgs D R
AB The major positive regulatory activity of the human alpha-globin gene complex has been localized to an element associated with a strong erythroid-specific DNase I hypersensitive site (**HS -40**) located 40 kb upstream of the **zeta** 2-globin mRNA cap site. Footprint and gel shift analyses of the element have demonstrated the presence of four binding sites for the nuclear factor GATA-1 and two sites corresponding to the AP-1 consensus binding sequence. This region resembles one of the major elements of the beta-globin locus control region in its constitution and characteristics; this together with evidence from expression studies suggests that **HS -40** is a primary element controlling alpha-globin gene expression.

L4 ANSWER 4 OF 21 MEDLINE
AN 94068584 MEDLINE
TI Analysis of the human alpha-globin gene cluster in transgenic mice.
SO PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1993 Dec 1) 90 (23) 11262-6.
Journal code: 7505876. ISSN: 0027-8424.
AU Sharpe J A; Wells D J; Whitelaw E; Vyas P; Higgs D R; Wood W G

AB A 350-bp segment of DNA associated with an erythroid-specific DNase I-hypersensitive site (**HS-40**), upstream of the alpha-globin gene cluster, has been identified as the major tissue-specific regulator of the alpha-globin genes. However, this element does not direct copy number-dependent or developmentally stable expression of the human genes in transgenic mice. To determine whether additional upstream hypersensitive sites could provide more complete regulation of alpha gene expression we have studied 17 lines of transgenic mice bearing various DNA fragments containing **HSs** -33, -10, -8, and -4, in addition to **HS -40**. Position-independent, high-level expression of the human **zeta**- and alpha-globin genes was consistently observed in embryonic erythroid cells. However, the additional **HSs** did not confer copy-number dependence, alter the level of expression, or prevent the variable down-regulation of expression in adults. These results suggest that the region upstream of the human alpha-globin genes is not equivalent to that upstream of the beta locus and that although the two clusters are coordinately expressed, there may be differences in their regulation.

L4 ANSWER 5 OF 21 MEDLINE
AN 93204975 MEDLINE
TI Transcriptional activation of human **zeta** 2 globin promoter by the alpha globin regulatory element (**HS-40**): functional role of specific nuclear factor-DNA complexes.
SO MOLECULAR AND CELLULAR BIOLOGY, (1993 Apr) 13 (4) 2298-308. Journal code: 8109087. ISSN: 0270-7306.
AU Zhang Q; Reddy P M; Yu C Y; Bastiani C; Higgs D; Stamatoyannopoulos G; Papayannopoulou T; Shen C K
AB We studied the functional interaction between human embryonic **zeta** 2 globin promoter and the alpha globin regulatory element (**HS-40**) located 40 kb upstream of the **zeta** 2 globin gene. It was shown by transient expression assay that **HS-40** behaved as an authentic enhancer for high-level **zeta** 2 globin promoter activity in K562 cells, an erythroid cell line of embryonic and/or fetal origin. Although sequences located between -559 and -88 of the **zeta** 2 globin gene were dispensable for its expression on enhancerless plasmids, they were required for the **HS-40** enhancer-mediated activity of the **zeta** 2 globin promoter. Site-directed mutagenesis demonstrated that this **HS-40** enhancer-**zeta** 2 globin promoter interaction is mediated by the two GATA-1 factor binding motifs located at -230 and -104, respectively. The functional domains of **HS-40** were also mapped. Bal 31 deletion mapping data suggested that one GATA-1 motif, one GT motif, and two NF-E2/AP1 motifs together formed the functional core of **HS -40** in the erythroid-specific activation of the **zeta** 2 globin promoter. Site-directed mutagenesis further demonstrated that the enhancer function of one of the two NF-E2/AP1 motifs of **HS-40** is mediated through its binding to NF-E2 but not AP1 transcription factor. Finally, we did genomic footprinting of the **HS-40** enhancer region in K562 cells, adult nucleated erythroblasts, and different nonerythroid cells. All sequence motifs within the functional core of **HS-40**, as mapped by transient expression analysis, appeared to bind a nuclear factor(s) in living K562 cells but not in nonerythroid cells. On the other hand, only one of the apparently nonfunctional sequence motifs was bound with factors in vivo. In comparison to K562, nucleated erythroblasts from adult human bone marrow exhibited a similar but nonidentical pattern of nuclear factor binding in vivo at the **HS-40** region. These data suggest that transcriptional activation of human embryonic **zeta** 2 globin gene and the fetal/adult alpha globin genes is mediated by erythroid cell-specific and developmental stage-specific nuclear factor-DNA complexes which form at the enhancer (**HS-40**) and the globin promoters.

L4 ANSWER 6 OF 21 MEDLINE
AN 95023182 MEDLINE
TI Analysis of a 70 kb segment of DNA containing the human **zeta** and alpha-globin genes linked to their regulatory element (**HS-40**) in transgenic mice.
SO NUCLEIC ACIDS RESEARCH, (1994 Oct 11) 22 (20) 4139-47.

Journal code: 0411011. ISSN: 0305-1048.
AU Gourdon G; Sharpe J A; Wells D; Wood W G; Higgs D R
AB We have ligated two cosmids through an oligonucleotide linker to produce a single fragment spanning 70 kb of the human alpha-globin cluster, in which the alpha-like globin genes (**zeta** 2, alpha 2 and alpha 1), their regulatory element (**HS-40**) and erythroid-specific DNase I hypersensitive sites accurately retain their normal genomic organization. The **zeta** (embryonic) and alpha (embryonic, fetal and adult) globin genes were expressed in all 17 transgenic embryos. Similarly, all fetal and adult mice from seven transgenic lines that contained one or more copies of the fragment, produced up to 66% of the level of endogenous mouse alpha-globin mRNA. However, as for smaller constructs containing these elements, human alpha-globin expression was not copy number dependent and decreased by 1.5-9.0 fold during development. These findings suggest that either it is not possible to obtain full regulation of human alpha-globin expression in transgenic mice or, more likely, that additional alpha-globin regulatory elements lie beyond the 70 kb segment of DNA analysed.

L4 ANSWER 7 OF 21 MEDLINE
AN 95327665 MEDLINE
TI Transcriptional activation of human adult alpha-globin genes by hypersensitive site-40 enhancer: function of nuclear factor-binding motifs occupied in erythroid cells.
SO PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1995 Jul 3) 92 (14) 6454-8.
Journal code: 7505876. ISSN: 0027-8424.
AU Rombel I; Hu K Y; Zhang Q; Papayannopoulou T; Stamatoyannopoulos G; Shen C K
AB The developmental stage- and erythroid lineage-specific activation of the human embryonic **zeta**- and fetal/adult alpha-globin genes is controlled by an upstream regulatory element [hypersensitive site (**HS**)-**40**] with locus control region properties, a process mediated by multiple nuclear factor-DNA complexes. In vitro DNase I protection experiments of the two G+C-rich, adult alpha-globin promoters have revealed a number of binding sites for nuclear factors that are common to HeLa and K-562 extracts. However, genomic footprinting analysis has demonstrated that only a subset of these sites, clustered between -130 and +1, is occupied in an erythroid tissue-specific manner. The function of these in vivo-occupied motifs of the alpha-globin promoters, as well as those previously mapped in the **HS-40** region, is assayed by site-directed mutagenesis and transient expression in embryonic/fetal erythroid K-562 cells. These studies, together with our expression data on the human embryonic **zeta**-globin promoter, provide a comprehensive view of the functional roles of individual nuclear factor-DNA complexes in the final stages of transcriptional activation of the human alpha-like globin promoters by the **HS-40** element.

L4 ANSWER 9 OF 21 MEDLINE
AN 95238333 MEDLINE
TI Functional roles of in vivo footprinted DNA motifs within an alpha-globin enhancer. Erythroid lineage and developmental stage specificities.
SO JOURNAL OF BIOLOGICAL CHEMISTRY, (1995 Apr 14) 270 (15) 8501-5.
Journal code: 2985121R. ISSN: 0021-9258.
AU Zhang Q; Rombel I; Reddy G N; Gang J B; Shen C K
AB Transcriptional regulation of the human alpha-like globin genes, embryonic **zeta** 2 and adult alpha, during erythroid development is mediated by a distal enhancer, **HS-40**. Previous protein-DNA binding studies have shown that **HS-40** consists of multiple nuclear factor binding motifs that are occupied in vivo in an erythroid lineage- and developmental stage-specific manner. We have systematically analyzed the functional roles of these factor binding motifs of **HS-40** by site-directed mutagenesis and transient expression assay in erythroid cell cultures. Three of these **HS-40** enhancer motifs, 5'NF-E2/AP1, GT II, and GATA-1(c), positively regulate the **zeta** 2-globin promoter activity in embryonic/fetal erythroid K562 cells and the adult alpha-globin promoter activity in adult erythroid MEL cells. On the other hand, the 3'NF-E2/AP1 motif is able to exert both positive and negative

regulatory effects on the **zeta** 2-globin promoter activity in K562 cells, and this dual function appears to be modulated through differential binding of the ubiquitous AP1 factors and the erythroid-enriched NF-E2 factor. Mutation in the GATA-1(d) motif, which exhibits an adult erythroid-specific genomic footprint, decreases the **HS-40** enhancer function in dimethyl sulfoxide-induced MEL cells but not in K562 cells. These studies have defined the regulatory roles of the different **HS-40** motifs. The remarkable correlation between genomic footprinting data and the mutagenesis results also suggests that the erythroid lineage- and developmental stage-specific regulation of human alpha-like globin promoters is indeed modulated by stable binding of specific nuclear factors in vivo.

L4 ANSWER 10 OF 21 CAPLUS COPYRIGHT 2003 ACS
AN 1996:128940 CAPLUS
DN 124:166363
TI Transcriptional regulation of human .zeta.2 and .alpha. globin promoters by multiple nuclear factor-DNA complexes: The final act
SO Molecular Biology of Hemoglobin Switching, Proceedings of the Conference on Hemoglobin Switching, 9th, Orcas Island, Wash., June 10-14, 1994 (1995), Meeting Date 1994, 193-202. Editor(s): Stamatoyannopoulos, George. Publisher: Intercept, Andover, UK.
CODEN: 62JIAN
AU Zhang, Qingyi; Rombel, Irene; Reddy, G. Narender; Shen, C. -K. James
AB A review, with 29 refs. Site-directed mutagenesis and transient expression assay were used to analyze functional contributions of individual nuclear factor-binding motifs to the transcriptional regulation of the two human .alpha.-like globin promoters, embryonic .**zeta**.2, and adult .alpha., by the **HS-40** element in embryonic/fetal erythroid K562 cells and adult erythroid MEL cells.

L4 ANSWER 12 OF 21 MEDLINE
AN 97086677 MEDLINE
TI Proximal promoter elements of the human zeta-globin gene confer embryonic-specific expression on a linked reporter gene in transgenic mice.
SO NUCLEIC ACIDS RESEARCH, (1996 Nov 1) 24 (21) 4158-64.
Journal code: 0411011. ISSN: 0305-1048.
AU Pondel M D; Sharpe J A; Clark S; Pearson L; Wood W G; Proudfoot N J
AB We have investigated the transcriptional regulation of the human embryonic **zeta**-globin gene promoter. First, we examined the effect that deletion of sequences 5' to **zeta**-globin's CCAAT box have on **zeta**-promoter activity in erythroid cell lines. Deletions of sequences between -116 and -556 (cap = 0) had little effect while further deletion to -84 reduced **zeta**-promoter activity by only 2-3-fold in both transiently and stably transfected erythroid cells. Constructs containing 67, 84 and 556 bp of **zeta**-globin 5' flanking region linked to a beta-galactosidase reporter gene (lacZ) and hypersensitive site -40 (**HS-40**) of the human alpha-globin gene cluster were then employed for the generation of transgenic mice. LacZ expression from all constructs, including a 67 bp **zeta**-globin promoter, was erythroid-specific and most active between 8.5 and 10.5 days post-fertilisation. By 16.5 days gestation, lacZ expression dropped 40-100-fold. These results suggest that embryonic-specific activation of the human **zeta**-globin promoter is conferred by a 67 bp **zeta**-promoter fragment containing only a CCAAT and TATA box.

L4 ANSWER 15 OF 21 MEDLINE
AN 1999061925 MEDLINE
TI Derepression of human embryonic zeta-globin promoter by a locus-control region sequence.
SO PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1998 Dec 8) 95 (25) 14669-74.
Journal code: 7505876. ISSN: 0027-8424.
AU Huang B L; Fan-Chiang I R; Wen S C; Koo H C; Kao W Y; Gavva N R; Shen C K
AB A multiple protein-DNA complex formed at a human alpha-globin locus-specific regulatory element, **HS-40**, confers appropriate developmental expression pattern on human embryonic **zeta**-globin promoter activity in humans and transgenic mice. We show here that introduction of a 1-bp mutation in an NF-E2/AP1 sequence

motif converts **HS-40** into an erythroid-specific locus-control region. Cis-linkage with this locus-control region, in contrast to the wild-type **HS-40**, allows erythroid lineage-specific derepression of the silenced human **zeta**-globin promoter in fetal and adult transgenic mice. Furthermore, **zeta** -globin promoter activities in adult mice increase in proportion to the number of integrated DNA fragments even at 19 copies/genome. The mutant **HS-40** in conjunction with human **zeta**-globin promoter thus can be used to direct position-independent and copy number-dependent expression of transgenes in adult erythroid cells. The data also supports a model in which competitive DNA binding of different members of the NF-E2/AP1 transcription factor family modulates the developmental stage specificity of an erythroid enhancer. Feasibility to reswitch on embryonic/fetal globin genes through the manipulation of nuclear factor binding at a single regulatory DNA motif is discussed.

L4 ANSWER 18 OF 21 MEDLINE
AN 2000153760 MEDLINE
TI Loading of DNA-binding factors to an erythroid enhancer.
SO MOLECULAR AND CELLULAR BIOLOGY, (2000 Mar) 20 (6) 1993-2003.
Journal code: 8109087. ISSN: 0270-7306.
AU Wen S C; Roder K; Hu K Y; Rombel I; Gavva N R; Daftari P; Kuo Y Y; Wang C; Shen C K
AB The **HS-40** enhancer is the major cis-acting regulatory element responsible for the developmental stage- and erythroid lineage-specific expression of the human alpha-like globin genes, the embryonic **zeta** and the adult alpha2/alpha/1. A model has been proposed in which competitive factor binding at one of the **HS-40** motifs, 3'-NA, modulates the capability of **HS-40** to activate the embryonic **zeta**-globin promoter. Furthermore, this modulation was thought to be mediated through configurational changes of the **HS-40** enhanceosome during development. In this study, we have further investigated the molecular basis of this model. First, human erythroid K562 cells stably integrated with various **HS-40** mutants cis linked to a human alpha-globin promoter-growth hormone hybrid gene were analyzed by genomic footprinting and expression analysis. By the assay, we demonstrate that factors bound at different motifs of **HS-40** indeed act in concert to build a fully functional enhanceosome. Thus, modification of factor binding at a single motif could drastically change the configuration and function of the **HS-40** enhanceosome. Second, a specific 1-bp, GC-->TA mutation in the 3'-NA motif of **HS-40**, 3'-NA(II), has been shown previously to cause significant derepression of the embryonic **zeta**-globin promoter activity in erythroid cells. This derepression was hypothesized to be regulated through competitive binding of different nuclear factors, in particular AP1 and NF-E2, to the 3'-NA motif. By gel mobility shift and transient cotransfection assays, we now show that 3'-NA(II) mutation completely abolishes the binding of small MafK homodimer. Surprisingly, NF-E2 as well as AP1 can still bind to the 3'-NA(II) sequence. The association constants of both NF-E2 and AP1 are similar to their interactions with the wild-type 3'-NA motif. However, the 3'-NA(II) mutation causes an approximately twofold reduction of the binding affinity of NF-E2 factor to the 3'-NA motif. This reduction of affinity could be accounted for by a twofold-higher rate of dissociation of the NF-E2-3'-NA(II) complex. Finally, we show by chromatin immunoprecipitation experiments that only binding of NF-E2, not AP1, could be detected in vivo in K562 cells around the **HS-40** region. These data exclude a role for AP1 in the developmental regulation of the human alpha-globin locus via the 3'-NA motif of **HS-40** in embryonic/fetal erythroid cells. Furthermore, extrapolation of the in vitro binding studies suggests that factors other than NF-E2, such as the small Maf homodimers, are likely involved in the regulation of the **HS-40** function in vivo.

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